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Cloning, Sequencing, and Expression in *Escherichia coli* of *lcnB*, a Third Bacteriocin Determinant from the Lactococcal Bacteriocin Plasmid p9B4-6

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On the bacteriocin plasmid p9B4-6 of *Lactococcus lactis* subsp. *cremoris* 9B4, a third bacteriocin determinant was identified. The genes encoding bacteriocin production and immunity resided on a 1.2-kb *CelII-ScaI* fragment and were located adjacent to one of two previously identified bacteriocin operons (M. J. van Belkum, B. J. Hayema, R. E. Jeeninga, J. Kok, and G. Venema, Appl. Environ. Microbiol. 57:492-498, 1991). The fragment was sequenced and analyzed by deletion and mutation analyses. The bacteriocin determinant consisted of two genes which were transcribed as an operon. The first gene (*lcnB*), containing 68 codons, was involved in bacteriocin activity. The second gene (*lciB*) contained 91 codons and was responsible for immunity. The specificity of this novel bacteriocin, designated lactococcin B, was different from that of the other two bacteriocins specified by p9B4-6. Part of the nucleotide sequence of the lactococcin B operon was similar to a nucleotide sequence also found in the two other bacteriocin operons of p9B4-6. This conserved region encompassed a nucleotide sequence upstream of the bacteriocin gene and the 5' part of the gene. When the lactococcin B operon was expressed in *Escherichia coli* by using a T7 RNA polymerase-specific promoter, antagonistic activity could be detected.

Lactic acid bacteria produce a variety of substances with antimicrobial activity which are of potential interest for food fermentation and preservation. In many cases, it has been shown that the compounds responsible for antagonistic activity, termed bacteriocins, were of a proteinaceous nature (16). Although numerous reports on the detection and biochemical characterization of bacteriocins produced by lactic acid bacteria are available, knowledge concerning their genetics is scarce. The genetic determinants for bacteriocin activity were shown to be plasmid encoded in several species (7, 8, 15, 22, 24, 25, 29), whereas other members of the lactic acid bacteria carried the bacteriocin genes on their chromosomes (1, 9, 13, 34). A number of bacteriocin determinants have recently been cloned. Scherwitz-Harmon and McKay (29) located the bacteriocin determinants of *Lactococcus lactis* subsp. *lactis* WM4 on two *BclI* fragments from the 88-MDa plasmid pNP2. The structural genes of helveticin J from *Lactobacillus helveticus* 481 and lactacin F from *Lactobacillus acidophilus* 11088 have been cloned and sequenced (14, 24). Additionally, the structural gene for prenisin has been cloned and sequenced from a number of lactococcal strains (4, 9, 15).

The genetic determinants for bacteriocin production and immunity in *L. lactis* subsp. *cremoris* 9B4 are located on the 60-kb conjugative plasmid p9B4-6 (25). From this plasmid, two fragments were cloned, each of which specified bacteriocin activity as well as immunity (35). A 1.8-kb *ScaI*-*Clal* fragment (contained in pMB225) specified low antagonistic activity, whereas the other, a 1.3-kb *ScaI*-*HindIII* fragment (present in pMB553), encoded high antagonistic activity. Sequence and mutation analyses (36) showed that the 1.8-kb fragment contained three open reading frames (ORFs); the first two (ORF-A1 and -A2) were involved in bacteriocin production, whereas the third (ORF-A3) was involved in

immunity. On the insert in pMB553, two ORFs were identified; the first one (ORF-B1) was responsible for bacteriocin activity, and the second (ORF-B2) specified immunity. On both fragments, the ORFs were organized in an operon. The specificities of the two bacteriocins were different. Recently, Holo et al. (12) have purified a bacteriocin, designated lactococcin A, from a strain of *L. lactis* subsp. *cremoris* and determined its amino acid sequence. The bacteriocin structural gene was cloned and sequenced with synthetic oligonucleotides. It appeared that the sequence was identical to the sequence of ORF-B1 of pMB553 and that the N-terminal part of the polypeptide specified by this ORF is cleaved off to give the mature bacteriocin molecule. To prevent confusion, we decided to rename ORF-B1 (the structural gene for lactococcin A) and -B2 (specifying immunity towards lactococcin A) *lcnA* and *lciA*, respectively. ORF-A1, -A2 (specifying a different type of bacteriocin activity), and -A3 (specifying the corresponding immunity) of pMB225 will now be designated *lcnM*, *lcnN*, and *lciM*, respectively. The bacteriocin specified by this plasmid will be designated lactococcin M.

Here we report on the cloning and sequencing of a third bacteriocin determinant of p9B4-6, specifying a bacteriocin designated lactococcin B, and show that the genes involved in bacteriocin production and immunity are also in this case transcribed as an operon. Using the T7 RNA polymerase-specific promoter for transcription of this operon in *Escherichia coli*, we were able to detect antagonistic activity by this host against sensitive lactococcal strains.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used are listed in Table 1. *E. coli* was grown in TY broth (26) and plated on TY agar medium. Glucose-M17 broth (33) and glucose-M17 agar were used to culture *L. lactis*. Selective concentrations of kanamycin,

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description ^a	Reference
Strains		
<i>E. coli</i>		
MH1	MC1061 derivative; <i>araD139 lacX74 galU</i> <i>galK hsr hsm⁺ strA</i>	5
BL21(DE3)	F ⁻ <i>ompT r_B⁻ m_B⁻ int</i> ; bacteriophage DE3 lysogen carrying the T7 RNA polymerase gene controlled by the <i>lacUV5</i> promoter	31
<i>L. lactis</i> subsp. <i>lactis</i> IL1403	Plasmid free	6
Plasmids		
pT712	Amp ^r , 2.8 kb; T7- polymerase expression vector	32
pMB225	Em ^r , 6.2 kb; specifying lactococcin M	35
pMB553	Em ^r , 5.1 kb; specifying lactococcin A	35
pMB500	Km ^r , 18.2 kb; specifying lactococcin A and B	35
pGKV210	Em ^r , 4.4 kb	38
pMB510	Km ^r , 16.1 kb	This study
pMB570	Em ^r , 6.5 kb; specifying lactococcin A and B	This study
pMB580	Em ^r , 5.6 kb; LcnB ⁺ , LciB ⁺	This study
pMB581	Em ^r , 5.6 kb; LcnB ⁺ , LciB ⁻	This study
pMB582	Em ^r , 4.6 kb; LcnB ⁻ , LciB ⁻	This study
pMB583	Em ^r , 5.6 kb; LcnB ⁻ , LciB ⁺	This study
pMB585	Em ^r , 5.5 kb; LcnB ⁻ , LciB ⁺	This study
pT580	Amp ^r , 3.8 kb	This study
pT585	Amp ^r , 3.7 kb	This study

^a LcnB⁺, producing lactococcin B; LcnB⁻, not producing lactococcin B; LciB⁻ and LciB⁺, sensitivity and immunity to lactococcin B, respectively; Amp^r, Em^r, and Cm^r, resistance to ampicillin, erythromycin, and chloramphenicol, respectively.

erythromycin, and ampicillin used for *E. coli* were 50, 100, and 50 µg/ml, respectively. For *L. lactis*, kanamycin and erythromycin were used at final concentrations of 50 and 5 µg/ml, respectively.

Molecular cloning and DNA sequence analysis. Isolation of plasmid DNA from *E. coli* was done as described by Birnboim and Doly (3). With some modifications (37), the same method was used to isolate plasmid DNA from *L. lactis*. Restriction enzymes, the Klenow fragment of *E. coli* DNA polymerase I, and T4 DNA ligase were obtained from Boehringer (Mannheim, Germany) and were used according to the instructions of the supplier. DNA cloning and manipulation techniques were carried out essentially as described by Maniatis et al. (20). *E. coli* cells were made competent and were transformed by the method of Mandel and Higa (19). Electrotransformation of *L. lactis* was carried out as described by Leenhouts et al. (18), using a Bio-Rad gene pulser (Bio-Rad Laboratories, Richmond, Calif.). Nucleotide sequence analysis was performed by sequencing double-stranded DNA in two orientations by the dideoxy-chain method of Sanger et al. (27), using the T7 sequencing kit (Pharmacia, Uppsala, Sweden).

MicroGenie software (Beckman, Palo Alto, Calif.) was used to identify restriction sites in the nucleotide sequence. DNA was amplified with *Taq* DNA polymerase (Promega,

Madison, Wis.) by using a Bio-med thermocycler 60 (B. Braun, Melsungen, Germany). Synthetic oligonucleotides for sequencing and DNA amplification were made with an Applied Biosystems 381A DNA synthesizer (Applied Biosystems, Foster City, Calif.). A deletion primer of 56 residues (5'-TATATGGTCCAGCACTCATAACATACTGCAA GCTCATAATAATCTCCTTATTTTAA-3'), complementary to residues 332 to 353 and 414 to 447 of the DNA sequence (see Fig. 2), together with a 17-mer primer (5'-TTCAAAGTCTACCCTGA-3') complementary to a sequence in the noncoding strand upstream of the multiple cloning site of pMB580, were used for DNA amplification with pMB580 as template.

Bacteriocin assay. Screening of *L. lactis* transformants for bacteriocin activity has been described previously (35). *L. lactis* IL1403 was used as the indicator strain.

T7 RNA polymerase-directed expression in *E. coli*. A culture of *E. coli* BL21(DE3) was grown to an optical density at 600 nm of 0.3 and subsequently induced for expression of the T7 RNA polymerase gene by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG; final concentration, 0.4 mM). After 1 h of incubation at 37°C, rifampin was added to a final concentration of 200 µg/ml. After an additional incubation of 1 h, cells were collected by centrifugation, concentrated 25-fold in sample buffer (62.5 mM Tris-HCl [pH 6.8]), 17.5% [vol/vol] glycerol, 2% [wt/vol] sodium dodecyl sulfate [SDS], 5% [vol/vol] β-mercaptoethanol, and 0.006% bromophenol blue), heated to 100°C for 10 min, and subjected to polyacrylamide gel electrophoresis (PAGE) (30).

Assay for bacteriocin activity in tricine-SDS-polyacrylamide gels. Samples (20 µl) of cell extract (equivalent to 500 µl of cell culture) were subjected to tricine-SDS-PAGE (28) by using a Bio-Rad mini-protein II (Bio-Rad). The compositions of the stacking gel and the spacer gel were as described by Schägger and von Jagow (28). The separating gel contained a mixture of 16% (wt/vol) acrylamide and 0.5% (wt/vol) bisacrylamide (28). After electrophoresis, the gel was fixed in 20% isopropanol and 10% acetic acid and washed in deionized water as described by Bhunia et al. (2). The gel was subsequently placed on a glucose-M17 agar plate. After 15 min, an overlayer of 4 ml of soft glucose-M17 agar (0.7%), containing 100 µl of an overnight culture of *L. lactis* IL1403 as an indicator, was poured on the surface of the agar plate. After 15 h of incubation at 30°C, the plate was examined for zones of inhibition of growth. As a reference, 12.5 µl of the supernatant of a bacteriocin-producing late-exponential-phase culture of *L. lactis*, mixed in a 1:1 ratio with 2× sample buffer (30), was also applied to the gel.

RESULTS

Identification of a novel bacteriocin determinant on p9B4-6. As reported previously (36), preliminary sequence analysis showed that downstream of the bacteriocin operon on the 1.3-kb *ScaI*-*HindIII* fragment of pMB553 (Fig. 1) a sequence was present which was similar to the identical nucleotide sequences found upstream of the two bacteriocin operons of p9B4-6. This observation prompted us to investigate whether a third bacteriocin determinant, interrupted by the *HindIII* site on the 3' end of the 1.3-kb *ScaI*-*HindIII* fragment, was present downstream of the lactococcin A operon. The region downstream of the 1.3-kb fragment was obtained by cloning a 2.1-kb *ScaI* fragment from pMB500 into the *SmaI* site of the pWV01-derived (17) shuttle vector pGKV210, giving plasmid pMB570 (Fig. 1). *L. lactis* IL1403 containing this plasmid inhibited the growth of IL1403

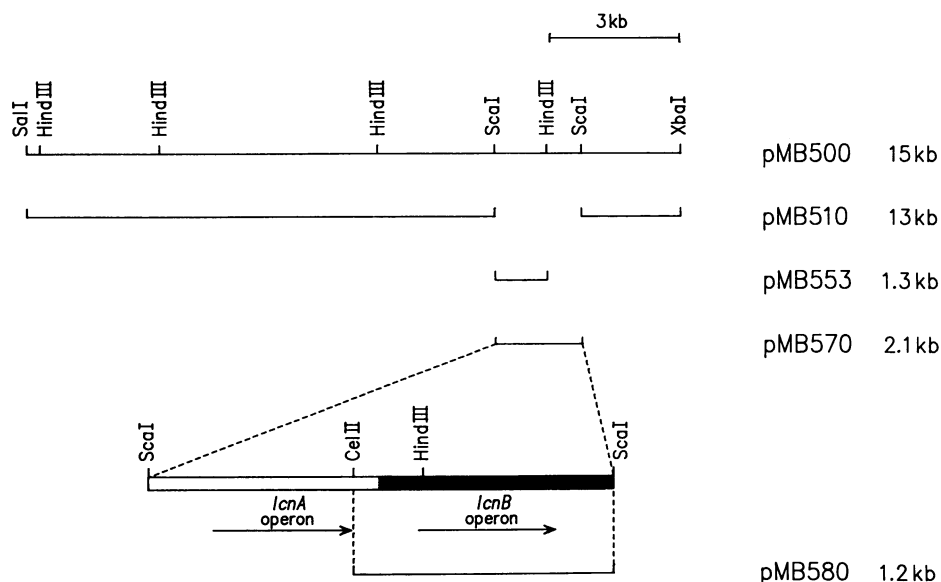


FIG. 1. Schematic representation of the insert in pMB500 and its derivatives. Only restriction enzyme sites relevant for the construction of the various subclones are shown. The arrows indicate the locations and the directions of transcription of the two bacteriocin operons present on pMB570. The part of the 2.1-kb *ScaI* fragment sequenced previously (36) is represented by the open bar. The sizes of the cloned fragments are indicated on the right.

(pMB553), but IL1403(pMB553) did not inhibit the growth of IL1403(pMB570). When IL1403 was used as the indicator strain, the size of the halo produced by IL1403(pMB570) was comparable to that produced by IL1403(pMB553) but somewhat reduced compared with that produced by IL1403 containing pMB500 (results not shown). When the 2.1-kb *ScaI* fragment was deleted from pMB500, resulting in plasmid pMB510 (Fig. 1), no antagonistic activity by *L. lactis* could be detected. The 2.1-kb *ScaI* fragment contains a unique *CelII* site which was used to delete a 0.9-kb *ScaI*-*CelII* fragment carrying the lactococcin A operon. *L. lactis* IL1403 carrying the resulting plasmid, pMB580 (Fig. 1), inhibited the growth of IL1403(pMB225) and IL1403 (pMB553) but was sensitive to the pMB225- and pMB553-specified bacteriocins. Apparently, we had succeeded in cloning a novel bacteriocin determinant that specified lactococcin B with a specificity different from that of the other two bacteriocins of p9B4-6.

Nucleotide sequence of the lactococcin B operon. The nucleotide sequence of the lactococcin B determinant present on the 2.1-kb *ScaI* fragment is shown in Fig. 2. Downstream of *lciA*, two ORFs were identified which could encode polypeptides of 68 and 91 amino acids, respectively. Both ORFs were preceded by a putative ribosome-binding site. An inverted repeat found 34 bp downstream of the TAA stop codon of the second ORF could act as a rho-independent terminator. The potential stem-and-loop structure had a ΔG of -82 kJ/mol. A stretch of 123 bp immediately upstream of the first ORF appeared to be almost identical to the nucleotide sequences upstream of the two other bacteriocin determinants of p9B4-6 (36), and therefore, the promoter sequences of the three bacteriocin operons are most probably identical (Fig. 2). Furthermore, the 5' nucleotide sequence of the first ORF was identical to the first 20 bp of *lcnM* and almost identical to the first 64 bp of *lcnA*. No further nucleotide sequence similarity between the three bacteriocin operons could be found. The amino acid sequences deduced

from both ORFs were compared with the deduced amino acid sequences from the genes of the two previously identified bacteriocin determinants of p9B4-6. Significant homology was found only between the N-terminal 21 amino acids of the polypeptides specified by the first ORF, *lcnA*, and *lcnM* (Fig. 3).

The lactococcin B operon contains genes for bacteriocin production and immunity. Both ORFs on the insert in pMB580 were subjected to in vitro deletion and mutagenesis. The mutated plasmid derivatives were isolated from *E. coli* MH1 and transferred to *L. lactis* IL1403, and the transformants were screened for bacteriocin production and immunity. Figure 4 shows the properties of the various pMB580 derivatives. Filling in the *AvaII* site with Klenow DNA polymerase resulted in the addition of an extra amino acid residue (Arg) between Gly-30 and Pro-31 in the first ORF (yielding plasmid pMB583). *L. lactis* cells containing this plasmid showed a $\text{Bac}^- \text{Imm}^+$ phenotype, which indicates that the first ORF, designated *lcnB*, is involved in bacteriocin production. Removal of the promoter region and the 5' part of *lcnB* by deleting the 0.31-kb *CelII*-*HindIII* fragment of pMB580 (pMB582) resulted in loss of bacteriocin activity as well as immunity, suggesting that the second ORF is involved in immunity and is transcribed from the same promoter as *lcnB*. Transformation of *L. lactis* with pMB581, which carries a frameshift mutation in the presumed immunity gene, by filling in the *BstNI* site of the second ORF gave rise to very small colonies of bacteriocin-producing transformants. When the growth medium was supplemented with 30% (vol/vol) supernatant of the bacteriocin-producing strain IL1403(pMB580), no transformants could be detected on the plates. Apparently, as was observed before (36), the initial concentration of bacteriocin produced by *L. lactis* transformants carrying a nonfunctional immunity gene was too low to completely inhibit the growth of the transformants. These results imply that the second ORF, designated *lciB*, indeed encodes immunity.

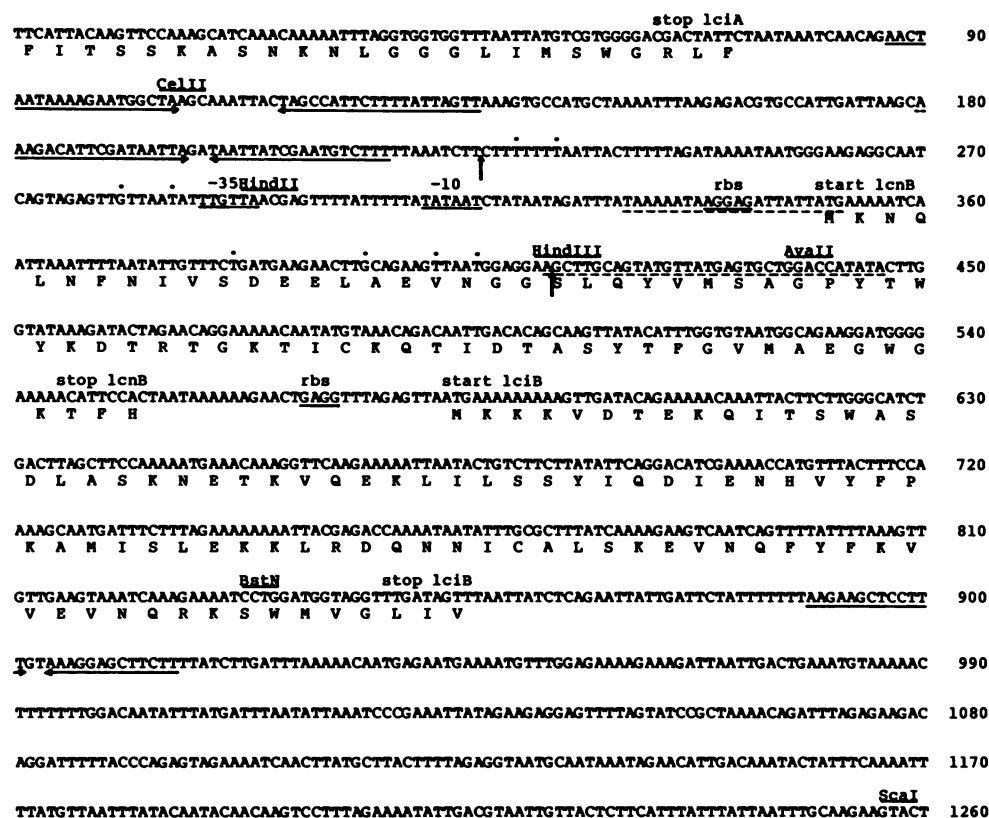


FIG. 2. Nucleotide and deduced amino acid sequences of a 1,260-bp segment of pMB570. The deduced amino acid sequences of *lcnB*, *lciB*, and the C-terminal part of *lciA* are shown below the nucleotide sequence. The putative -35 and -10 regions of the promoter as well as the putative ribosome-binding sites (rbs) of *lcnB* and *lciB* are indicated. The inverted repeats downstream of *lciA* and *lciB* are underlined by solid arrows. Restriction enzyme sites used for mutagenesis are indicated. The region between the two vertical arrows below the DNA sequence represents the region of similarity of the nucleotide sequence upstream of *lcnB* and the 5' part of *lcnB* with the sequence upstream of *lcnA* and the 5' part of *lcnA*. Mismatches are represented by dots above the nucleotide sequence. The two broken lines represent the sequence of the deletion primer used to remove the intervening sequence by polymerase chain reaction.

Lactococcin B production in *E. coli*. As reported for the two other bacteriocins of p9B4-6 (35), no antagonistic activity of lactococcin B in cells or lysates of *E. coli*(pMB580) could be observed. In an attempt to express lactococcin B in *E. coli*, the bacteriocin operon of pMB580 was introduced into the *E. coli* expression vector pT712. The 0.97-kb *HindII*-*EcoRI* fragment of pMB580 (the *Scal* site was lost as a consequence of the cloning, and therefore, the *EcoRI* site of the multiple cloning site was used) was cloned between the *HindII* and *EcoRI* sites of pT712. As the *HindII* site is located between the -35 and -10 regions (Fig. 2), this disrupted the promoter of the bacteriocin operon. In the resulting plasmid, pT580, the bacteriocin operon was placed under control of the T7 RNA polymerase-specific promoter. After induction of the T7 RNA polymerase gene, samples of

E. coli BL21(DE3) carrying pT580 were subjected to tricine-SDS-PAGE and subsequently examined for bacteriocin activity. At a position corresponding to about 6.5 kDa, a zone of inhibition of growth that was absent in samples of *E. coli* BL21(DE3) containing pT712 was observed (Fig. 5). The zone of inhibition produced by a sample of the supernatant of a culture of IL1403(pMB580) was found at a position corresponding to about 3.4 kDa (Fig. 5). Apparently, the size of the lactococcin B molecule secreted by the *L. lactis* cells is less than that of the polypeptide responsible for the antagonistic activity in the *E. coli* extracts. No bacteriocin activity in the supernatant of the *E. coli* culture containing pT580 could be detected. An extract of *E. coli* BL21(DE3) containing pMB580 did not give rise to antagonistic activity on the gel (Fig. 5, lane 4). Holo et al. (12) have shown that the bacteriocin encoded by *lcnA* is synthesized as a precursor of 75 amino acids and is processed by removal of the N-terminal 21 amino acids. As shown in Fig. 4, the first 21 amino acids of the *lcnM*, *lcnA*, and *lcnB* products are nearly identical, which strongly suggests that these polypeptides are processed at the same position. This assumption seems to be confirmed by the fact that lactococcin A, with a precursor almost equal in size to the polypeptide specified by *lcnB*, migrates to the same position on the gel as lactococcin B (36). To examine whether the absence of the N-terminal

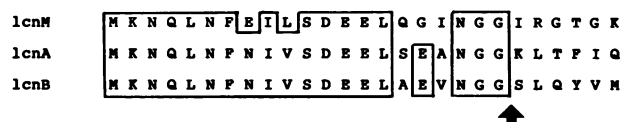


FIG. 3. Homology comparison of the N-terminal parts of the deduced amino acid sequences of *lcnM*, *lcnA*, and *lcnB*. Identical amino acids are boxed. The arrow indicates the processing site in the lactococcin A precursor as determined by Holo et al. (12).

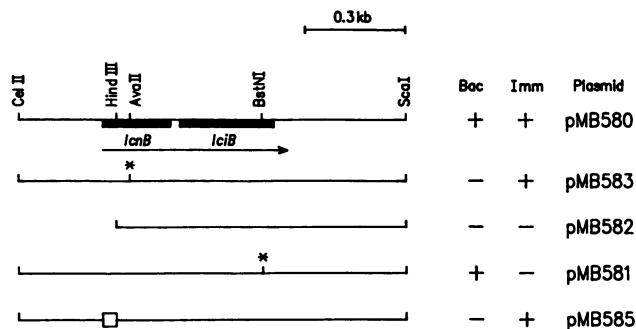


FIG. 4. Phenotype of various mutation derivatives of pMB580. The restriction enzyme map and location of *lcnB* and *lciB* on the 1.2-kb *ClaII-ScaI* fragment of pMB580 are shown. The lines represent the parts of the 1.2-kb fragment present in the various subclones. Asterisks indicate mutations in the ORFs (for details, see text). The arrow indicates the direction of transcription. Bacteriocin production (Bac) and immunity (Imm) expressed by the various clones are indicated. The open box represents the region deleted from *lcnB* by polymerase chain reaction.

part, which is apparently removed in *L. lactis*, had any effect on lactococcin B activity, the DNA sequence between nucleotides 353 and 414 was deleted from *lcnB* by polymerase chain reaction (see Materials and Methods). The polymerase chain reaction product was digested with *PstI* and *AvaII* and used to replace the *PstI-AvaII* fragment of pMB580, giving pMB585 (Fig. 4). This plasmid contained the expected deletion endpoints, as judged by nucleotide sequence analysis. *L. lactis* cells containing pMB585 grew normally and were Bac⁻ Imm⁺. When the 0.91-kb *HindIII-EcoRI* fragment of pMB585 was cloned in pT712, extracts of *E. coli* BL21(DE3) containing the resulting construct pT585 did not give rise to antagonistic activity as determined by tricine-SDS-PAGE (results not shown).

DISCUSSION

The work presented here describes the identification and molecular analysis of the genetic determinant of lactococcin B, a bacteriocin with a specificity different from that of the

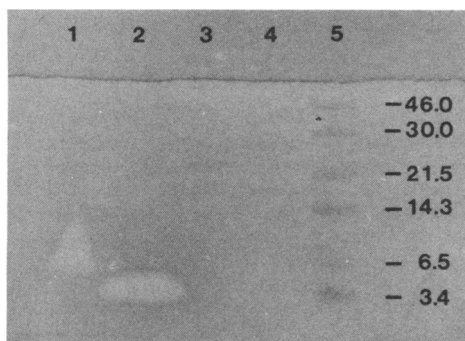


FIG. 5. Detection of bacteriocin activity in a tricine-SDS-polyacrylamide gel by the overlay test. Lanes: 1, 3, and 4, lysates of *E. coli* BL21(DE3) containing pT580, pT712, and pMB580, respectively; lane 2, supernatant of *L. lactis* IL1403 containing pMB580; lane 5, molecular size standard (Rainbow protein molecular weight marker; Amersham International, Amersham, England); molecular sizes (in kilodaltons) are shown on the right.

bacteriocins specified by the two previously cloned bacteriocin determinants of p9B4-6 (35).

We have reported previously that *L. lactis* cells containing pMB500 showed a higher level of bacteriocin activity than *L. lactis* cells carrying pMB553 (35). A subclone (pMB570) carrying the 2.1-kb *ScaI* fragment of pMB500 had a level of bacteriocin activity still less than that of pMB500. Inhibition studies with pMB510 showed that no further bacteriocin determinants were present on the 15-kb fragment. Apparently, some other information on pMB500 is responsible for optimal expression of the bacteriocin phenotype.

The reason why the nucleotide regions upstream of the genes of the bacteriocin operons and also the 5' ends of the first genes of these operons are similar is as yet unclear. Preliminary results indicate that immediately upstream of the promoter regions of the lactococcin M and A operons, the 3' end of an ORF is present. The fact that the three bacteriocin operons are linked to similar DNA regions may suggest that their combined presence on p9B4-6 is the result of recombinational events, thus providing the organism with a selective advantage in a competitive environment with other bacteriocin-producing lactococci.

Previous experiments to express bacteriocin activity in *E. coli* were unsuccessful (35). Attempts to express bacteriocin activity in *E. coli* also failed for helveticin J (14) and for the bacteriocin BCN5 of *Clostridium perfringens* (10). In both studies, it was suggested that the high A+T content of the genes involved was responsible for the lack of expression, as codons rich in A and T are infrequently used in *E. coli*. Using the T7 polymerase-specific promoter, we were able to detect antagonistic activity with the pMB580-specified bacteriocin in *E. coli*. The finding that the *lcnA* product is processed by removal of the 21-amino-acid N terminus (12) and the fact that this N terminus is similar to the N termini of the *lcnM* and *lcnB* products strongly suggest that the polypeptides encoded by *lcnM*, *lcnA*, and *lcnB* are processed in an identical way in *L. lactis*. The difference in molecular size between the bacteriocin present in the *L. lactis* supernatant and the protein responsible for antagonistic activity in the *E. coli* extract may be explained by assuming that *E. coli* is unable to process the bacteriocin precursor. Deletion of the 5' part of *lcnB* to obtain the mature-sized bacteriocin as the primary translation product did not result in activity in cells or lysates of *L. lactis* and *E. coli*. Either the truncated gene is poorly expressed or the N-terminal part is essential to get an active product. The molecular size of the primary translation product specified by *lcnB* is 7.6 kDa. After deletion of its 21 N-terminal amino acids, postulated on the basis of the similarity to lactococcin A, the mature product would be 5.3 kDa. The discrepancy between these values and the molecular sizes determined by tricine-SDS-PAGE is probably due to the hydrophobic nature of the polypeptide specified by *lcnB*. Muriana and Klaenhammer (23) observed a similar difference between the molecular size deduced from SDS-PAGE and that determined from the amino acid composition of the purified bacteriocin lactacin F.

Recently, the bacteriocin pediococcin PA-1 from *Pediococcus acidilactici* PAC 1.0 was purified and its amino acid sequence was determined (11, 21). Subsequently, the bacteriocin structural gene was cloned and sequenced, and it was shown that the bacteriocin molecule was synthesized as a precursor of 62 amino acids. An N-terminal sequence consisting of 18 amino acids was removed to yield the mature bacteriocin molecule. The cleavage occurred on the C-terminal side of two adjacent glycine residues (21). Lactacin F was also produced as a precursor with a leader peptide of 18

amino acids (24). Also in this case, cleavage occurred at the C-terminal side of two glycine residues, resulting in the formation of a mature peptide of 57 amino acids (24). As is shown in Fig. 3, a similar cleavage site behind two glycine residues was also found in the precursor of lactococcin A, and on the basis of similarity between their N termini, most probably this also applies to the lactococcin B and M precursors. Thus, it would seem that a common cleavage site is used in lactic acid bacteria and that a similar processing mechanism underlies maturation of a variety of small hydrophobic bacteriocins in these organisms.

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